Carbohydrate Polymers 112 (2014) 686–694

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Salt stress alters the cell wall polysaccharides and anatomy of coffee (*Coffea arabica* L.) leaf cells

Rogério Barbosa de Lima[a], Tiago Benedito dos Santos[b], Luiz Gonzaga Esteves Vieira[b], Maria de Lourdes Lúcio Ferrarese[c], Osvaldo Ferrarese-Filho[c], Lucélia Donatti[d], Maria Regina Torres Boeger[e], Carmen Lúcia de Oliveira Petkowicz[a,*]

[a] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, C.P.19046, Curitiba 81531-980, Brazil
[b] Laboratório de Biotecnologia Vegetal, Instituto Agronômico do Paraná, Londrina 86001-970, Brazil
[c] Departamento de Bioquímica, Universidade Estadual de Maringá, Maringá 87020-900, Brazil
[d] Departamento de Biologia Celular, Universidade Federal do Paraná, Curitiba 81531-980, Brazil
[e] Departamento de Botânica, Universidade Federal do Paraná, Curitiba 81531-980, Brazil

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form 10 June 2014
Accepted 11 June 2014
Available online 26 June 2014

Keywords:
Salt stress
Coffea arabica
Cell wall
Polysaccharide
Lignin

ABSTRACT

Coffea arabica is the most important agricultural commodity in the world, and salinity is a major threat to its sustainable irrigation. Coffee leaf polysaccharides from plants subjected to salt stress were extracted and the leaves visualized through optical and electron microscopy. Alterations were detected in the monosaccharide composition of the pectin and hemicelluloses, with increases in uronic acid in all fractions. Changes in the polysaccharides were confirmed by HPSEC and FTIR. Moreover, the monolignol content was increased in the final residue, which suggests increased lignin content. The cytoplasm was altered, and the chloroplasts appeared irregular in shape. The arrangement of the stroma lamellae was disordered, and no starch granules were present. It was concluded that leaves of *C. arabica* under salt stress showed alterations in cell wall polysaccharides, increased monolignol content and structural damage to the cells of the mesophyll.

1. Introduction

Plant cell walls are dynamic entities that govern the morphology, growth and development of plants (Albersheim et al., 1994). Cell walls also mediate interactions between the cell and its environment (Pennell, 1998), including environmental stresses such as wounding (Cardemil & Riquelme, 1991), mineral stress (Fernandes, Garcia-Angulo, Goulao, Acebes, & Amancio, 2013), osmotic stress (Wakabayashi, Hoson, & Kamisaka, 1997), cold acclimation (Domon et al., 2014; Weiser, Wallner, & Waddell, 1990), drought tolerance (Zwiazek, 1991) and salt stress (Zhong & Läuchli, 1993). Salt stress is a major threat to sustainable irrigation, which is required to meet the food demands of the increasing human population (Flowers, 2004). Salt stress can cause multifarious adverse effects on plant metabolism (Munns & Tester, 2008). However, there is little information regarding plant cell wall responses to salt stress.

In the mature organs of plant species, two types of cell walls can be present: primary and secondary plant cell walls. The primary walls are deposited during cell division and are typical of growing cells, while lignified secondary walls are deposited in some cell types after cell growth has ceased (Carpita & Gibeaut, 1993). Primary cell walls are composed of cellulose, pectins, hemicelluloses and protein and phenolic compounds. Primary cell walls generate turgor pressure, thus resisting tensile forces, in addition to accommodating cell expansion and mediating cell adhesion; these cell walls are found at the surface of all plant cells. Secondary cell walls are restricted to specific types of differentiated cells and are composites of cellulose and hemicelluloses, often being encrusted with lignin. Secondary cell walls are thicker than the primary walls and are resistant to compressive forces (Doblin, Pettolino, & Bacic, 2010). In leaves, cells with non-lignified primary walls include the palisade and spongy parenchyma and epidermal cells, whereas cells with lignified secondary walls often include tracheary elements and sclerenchyma (Taiz & Zeiger, 2006). The structure of plant cell walls and the ultrastructure of plant cells can be altered under conditions of biotic and abiotic stress (Bennici & Tani, 2009; Miyake, Mitsuya, & Rahman, 2006).

Salinity limits crop productivity in many areas of the world. Salts decrease water potential and create a water deficit problem for

* Corresponding author. Tel.: +55 41 3361 1661; fax: +55 41 3266 2042.
E-mail address: clop@ufpr.br (C.L. de Oliveira Petkowicz).

http://dx.doi.org/10.1016/j.carbpol.2014.06.042
0144-8617/

plant growth. The increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 15 years and causing up to 50% loss by the year 2050 (Wang, Vinocur, & Altman, 2003).

Coffee is an important crop, with more than 115 million tons of 60 kg bags being exported from March 2012 to February 2013 (ICO, 2014). Coffee production involves approximately half a million people in various processes, ranging from cultivation to the generation of the final consumable (Budzinski et al., 2010). It has been shown that salt stress affects the growth (Nazário, Garcia, Gonçalves, Madalão, & Araujo, 2010) and levels of raffinose family oligosaccharides (RFOs) in coffee plants (Dos Santos et al., 2011). RFOs are involved in osmoprotection (Dos Santos et al., 2011; Kerepesi & Galiba, 2000) and in reactive oxygen species (ROS) scavenging to combat oxidative damage caused by salinity (Nishizawa, Yabuta, & Shigeoka, 2008).

Recently, Lima et al. (2013) identified changes in cell wall polymers and the anatomy of coffee (*Coffea arabica*) leaves subjected to heat stress. However, there is little information regarding the cell wall polymers and ultrastructural responses of *C. arabica* to salt stress. Therefore, the objective of this study was to evaluate the cell wall polysaccharides and monolignol composition of *C. arabica* leaves subjected to salt stress conditions. Furthermore, because structural alterations in the leaf anatomy may play an important role during abiotic stress, the structure of mesophyll cells subjected to salt stress was also evaluated.

2. Materials and methods

2.1. Sample preparation

Coffea arabica cv. IAPAR-59 was cultivated under field conditions at the experimental station of the Agronomic Institute of Paraná (latitude 23°18′S, longitude 51°09′0, 585 m average altitude, Londrina, Brazil). Initially, each plant was irrigated daily and fertilized weekly with 100 mL of Hoagland's nutrient solution. The salt stress experiment was performed in a greenhouse at 25 °C with a 12 h/12 h day/night cycle. Six month-old plants were used, with one plant being grown per container. The containers (4 L) were equally filled with soil, sand and organic compound mixture (3:1:1). To avoid osmotic shock, these plants were irrigated on the first day with 50 mmol/L NaCl and on the second day with 100 mmol/L NaCl. From the third day until the end of the experiment, the plants were irrigated daily with a 150 mmol/L NaCl solution. A pair of leaves was collected from each of five plants at each of the following sampling times: before the beginning of the experiment (non-stressed control – Day 0) and on days 3, 6, 12 and 25 after starting the treatment with 150 mmol/L NaCl. After harvesting, all samples were frozen immediately in liquid nitrogen following removal of the main vein, then ground to a fine powder with a mortar and pestle and stored in a freezer until use.

2.2. Cell wall extraction

Lyophilized leaves were subjected to sequential treatments with chloroform and ethanol:water/7:3, according to the method of Albini et al. (1994), in order to remove the chlorophyll and low molar mass compounds, respectively. Polysaccharides were sequentially extracted from the leaves as follows: three extractions were performed with water at 80 °C for 5 h (fraction W), followed by two extractions with 2% EDTA at 30 °C for 5 h (fraction E), three extractions with 4 mol/L NaOH at 30 °C for 5 h (fraction H30) and one extraction with 4 mol/L NaOH at 70 °C for 3 h (fraction H70). The alkaline solutions contained $NaBH_4$ to prevent end peeling. The polysaccharides were precipitated with 3 volumes of ethanol, then stored overnight at 4 °C and subsequently isolated via centrifugation (8000 × *g*) and washed three times with ethanol. The EDTA and NaOH fractions were dialyzed for 48 h against tap water. All fractions were subjected to enzymatic treatment for starch removal using a previously reported protocol (Bacic, 2006). The polysaccharides were then treated with porcine α-amylase and amyloglucosidase (2 units/mg carbohydrate) for 8 h (40 °C) in Tris–maleate buffer containing 10 mmol/L NaCl and 1 mmol/L $CaCl_2$. After the digestion was complete, the polysaccharides were precipitated with ethanol, stored overnight at 4 °C and washed thoroughly with ethanol via centrifugation (8000 × *g*). The starch-free preparations were then dried under vacuum for further analyses.

2.3. Monosaccharide composition

The polysaccharides (200 μg) were hydrolyzed with 2 mol/L trifluoroacetic acid (0.5 mL, 5 h, 100 °C), evaporated to dryness using a Speed Vac vacuum centrifuge (Savant) and dissolved in water (0.5 mL). The monosaccharides were then reduced with $NaBH_4$ for 16 h at 25 °C. The resulting products were treated with 0.5 mL glacial acetic acid and evaporated to dryness. Following the addition of methanol (1 mL), the mixture was dried three times, and the residue was acetylated with absolute acetic anhydride P.A. for 30 min at 100 °C (Vinogradov & Wasser, 2005).

The resulting alditol acetates were analyzed via gas–liquid chromatography using a model 5890 S II Hewlett-Packard gas chromatograph at 220 °C with a flame ionization detector and an injector temperature of 250 °C. A DB-210 capillary column (0.25 mm internal diameter × 30 m) was used, with a film thickness of 0.25 μm. Nitrogen was employed as the carrier gas (2.0 mL/min).

The uronic acid content was estimated with the meta-hydroxydiphenyl colorimetric method (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as the standard. The insoluble fractions were evaluated after the samples were dissolved in sulfuric acid (Ahmed & Labavitch, 1977).

2.4. Monolignol composition

Alkaline nitrobenzene oxidation was employed to determine the monomeric composition of lignin (Zanardo, Lima, Ferrarese, Bubna, & Ferrarese-Filho, 2009) for the final insoluble residues (20 mg). The samples were sealed in a Pyrex® ampule containing 1 mL nitrobenzene P.A. and heated to 170 °C for 90 min. The sample was occasionally shaken during the course of the reaction. The sample was subsequently cooled to room temperature, washed twice with chloroform, acidified to pH 2 with 2 mol/L HCl and extracted twice with chloroform. The organic extracts were combined, dried, resuspended in 1 mL methanol and diluted in a mixture of methanol and 4% acetic acid in water (20:80, v/v). All samples were filtered through a 0.45 μm disposable syringe filter and analyzed via high performance liquid chromatography (HPLC) using a Shim-pack CLC-ODS (M) column (4.6 mm I.D. × 250 mm). The mobile phase consisted of a mixture of methanol and 4% acetic acid water (20:80, v/v), and a flow rate of 1.2 mL/min was used during an isocratic run of 20 min. Quantification of the monomeric aldehyde products *p*-hydroxybenzaldehyde, vanillin and syringaldehyde released through nitrobenzene oxidation was performed at 290 nm using corresponding standards. The results were expressed as μg monomer/mg final insoluble residue.

2.5. High-pressure size exclusion chromatography (HPSEC) analysis

A Waters high performance size exclusion chromatography (HPSEC) apparatus was coupled to a Waters 2410 differential

refractometer (RI) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection instrument. A solution of 0.1 mol/L $NaNO_2$ containing NaN_3 (0.5 g/L) was used as the eluent at a flux of 0.6 mL/min. Previously filtered samples (0.20 μm; Millipore) were analyzed at a concentration of 1.0 mg/mL, and data were collected using the Wyatt Technology ASTRA program.

2.6. Fourier transform-infrared spectroscopy (FTIR)

For FTIR analysis, the samples were dried in an Abderhalden apparatus and stored in desiccators. Pellets were prepared from mixtures of the samples with KBr at a 1:100 (w/w) ratio. The infrared spectra were collected on a Bomem Hartmann & Braun spectrometer over a range of 1800–900 cm^{-1} at a resolution of 4 cm^{-1} in absorbance mode. The spectra were averaged using 32 scans.

2.7. Electron and optical microscopy

The leaves were fixed with modified Karnovsky's fixative without calcium chloride and with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.2 cacodylic acid buffer (pH 7.2) (Karnovsky, 1965), then washed in 0.1 mol/L cacodylic acid buffer (pH 7.2) and post-fixed in 2% OsO_4 in 0.1 mol/L cacodylic acid buffer (pH 7.2) for 1 h. Subsequently, the leaves were dehydrated with ethanol and acetone, embedded in Epon 812 (Luft, 1961), contrasted using uranyl acetate and lead citrate and examined with a JEOL-JEM 1200 EX II transmission electron microscope at an accelerating voltage of 100 kV (Peabody, MA, USA). The leaf blades were fixed with Karnovsky's fixative, embedded in metacrilatoaglicol (JB-4) and sectioned on a rotary microtome. Transverse sections (60–100 nm) were stained with 0.05% toluidine blue (Feder & O'Brien, 1968), mounted in synthetic resin (Entellan®) and observed and photographed under an OLYMPUS BH-2 optical microscope.

2.8. Statistical design

The experimental design was completely randomized, and each experiment involved at least 5 plants. The biological samples consisted of pools of coffee plant leaves collected at the same developmental stage and the work was conducted using technical replicates. The data from monosaccharide and monolignol composition are expressed as the mean of three independent experiments ± S.E. A one-way variance analysis for testing the significance of the observed differences was performed using Prism® (Version 5.0). Differences between the parameters were evaluated using the Tukey test, and P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Analysis of the yield of cell wall polysaccharides from coffee leaves following saline stress

The cell wall can be extracted with hot water and either a chelating agent or dilute acid, yielding the pectin fraction. Following the removal of pectin, the remaining cell wall polysaccharides can be extracted using alkaline solutions, yielding the hemicellulose fraction, which extraction yield depends on the temperature (N'Diaye & Rigal, 2000). The cell wall residue, which remains insoluble after extraction with alkali, contains the cellulose microfibrils (Brett & Waldron, 1990). The polysaccharide yields obtained through the sequential extraction of cell wall components from the leaves of control and salt-stressed coffee plants are shown in Table 1. The highest yields were observed for the hemicellulosic fraction extracted with 4 mol/L NaOH at 30 °C (23.6–11.5%). The pectins, fractions W and E, exhibited lower yields (2.4–7.3%). The total content of hemicelluloses extracted in fractions H30 and H70 ranged from 14.5% to 25.6%, whereas values of 5.6% to 11.1% were observed for the total amount of pectic polysaccharides.

Table 1
Yields[a] of the cell wall fractions obtained from *Coffea arabica* leaves after 0 (control), 3, 6, 12 and 25 days under salt stress (treatment with 150 mmol/L NaCl).

Days/fraction	Pectins		Hemicelluloses		Cellulose
	W (%)	E (%)	H30 (%)	H70 (%)	R (%)
Day 0 (control)	7.3	3.3	23.6	2.0	11.7
Day 3	3.2	2.4	17.3	4.2	17.1
Day 6	4.4	5.9	14.6	4.2	15.2
Day 12	5.7	5.4	11.5	3.0	17.0
Day 25	3.9	5.5	14.5	2.6	17.8

[a] Expressed as percentages of the total leaf based on the insoluble residues obtained through sequential treatment with chloroform and methanol:water/7:3.

The extractability of the pectin and hemicellulosic fractions was altered by salt stress. Salt stress resulted in a decrease in the content of water-extractable pectins, whereas the amount of pectins extracted with the chelating agent increased compared to the control plants.

The hemicellulosic fractions extracted at 30 °C from plants subjected to salt stress exhibited lower yields than control plants. The H70 fraction displayed lower yields than the H30 fractions, and following salt stress, the yield was increased compared to the control. However, a decrease between day 12 and 25 was observed for H70 fraction and the total yield of hemicellulose (H30 + H70) from plants subjected to salt stress was decreased compared to the control.

The differences in the extractability of polysaccharides following salt stress resulted in an increased amount of insoluble cell wall material (fraction lignocellulosic) under salt stress. The increase in the amount of insoluble cell wall material may be due to a stronger association of polymers under salt stress. Therefore, these results suggest that saline stress induces changes in the organization of cell polymers, thereby modifying the extractability of cell wall polysaccharides.

3.2. Analysis of pectins from cell walls of coffee leaves following saline stress

The changes that occur in water-soluble pectins from plants subjected to saline stress were analyzed by comparing their monosaccharide composition with the control group (Fig. 1A). Arabinose, galactose, glucose and uronic acid were the major monosaccharides in the W fractions obtained following the different treatments, suggesting the presence of arabinogalactans and acidic pectins. Xylose, mannose and rhamnose were also detected. The amount of uronic acid increased in the fractions isolated from plants subjected to salt stress.

The size of polysaccharides represents important information related to the alteration of cell wall components (Zhong & Läuchli, 1993). The elution profiles obtained through HPSEC using the RI detector showed differences between the water-soluble pectins from plants subjected to salt stress conditions compared to the control group (Fig. 1B). All fractions displayed a polymodal mass distribution. Following 12 days of salt stress, the main peak (~52 min) in the control sample was shifted to a higher elution time. In addition, along the salt stress gradient, the peak eluting after 60 min became more defined and intense.

In the "fingerprint" region of the FTIR spectrum (Fig. 1C), bands near 1100 cm^{-1} indicate several different modes, such as C—H bending or C—O or C—C stretching. Absorbances observed at 1076 and 1043 cm^{-1} are typical of arabinogalactans (Kačuráková,

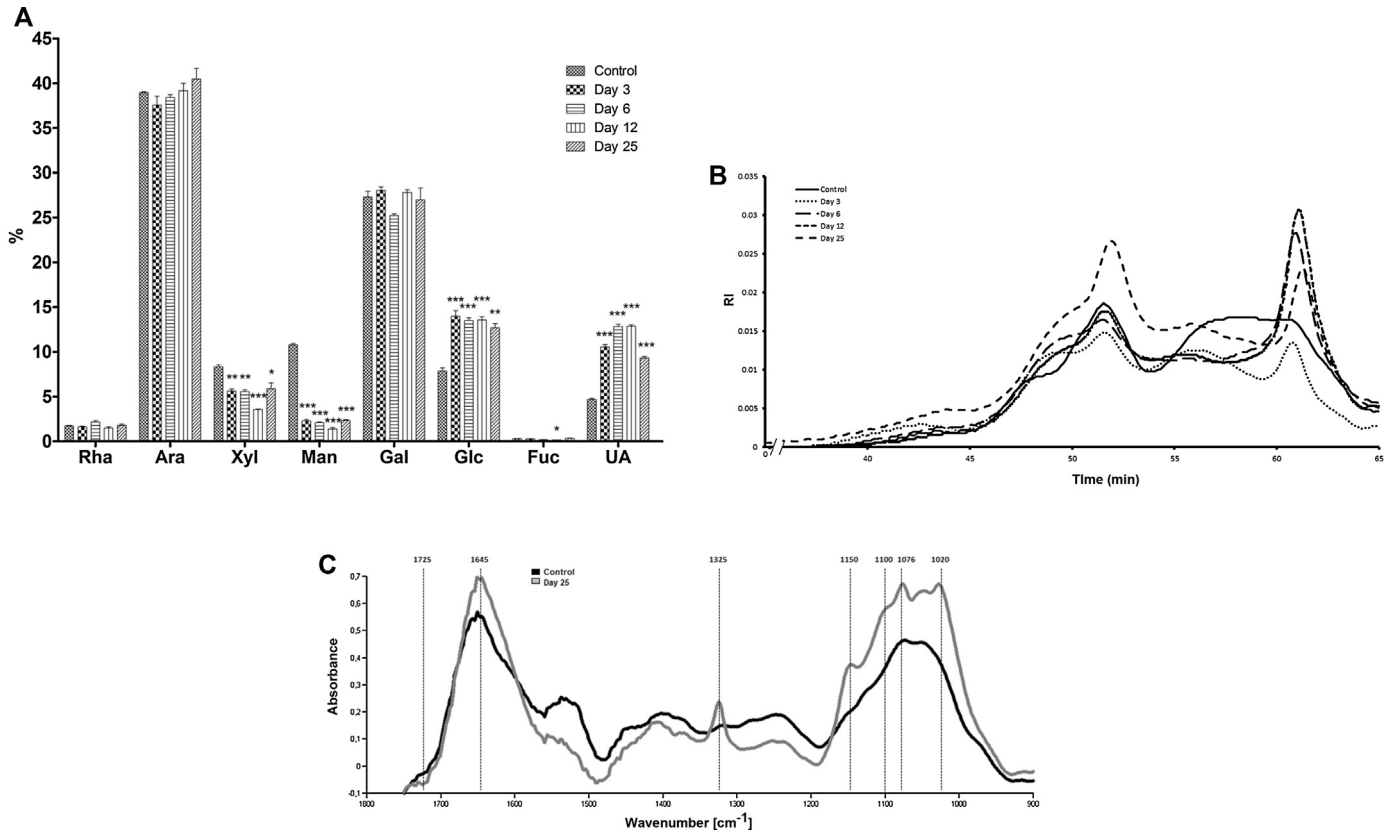

Fig. 1. Monosaccharide composition (A); HPSEC analysis with RI detection (B); and the FTIR spectra (control and after 25 days of salt stress) (C) of the W fractions from *Coffea arabica* control leaves and leaves subjected to salt stress. Rha, rhamnose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; Fuc, fucose, UA, uronic acid. Each value represents the mean of three determinations. The error bars indicate the SE. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Capek, Sasinková, Wellner, & Ebringerová, 2000). The bands associated with homogalacturonan (at 1645; 1325; 1150; 1100 and 1020 cm^{-1}) were increased in the fraction extracted from plants subjected to salt stress (Recio, 2003; Wilson et al., 2000). These data are consistent with the results of the monosaccharide composition analysis and suggest that salt stress causes changes in the pectins in the cell walls of coffee leaves. Following the removal of starch from the E fractions, there was not sufficient material remaining to perform any analysis.

3.3. Analysis of hemicelluloses from the cell walls of coffee leaves following saline stress

The influence of saline stress on coffee leaf hemicelluloses was evaluated by analyzing the composition of the polysaccharide fractions. The qualitative components of hemicellulose fractions H30 (Fig. 2A) and H70 (Fig. 2B) were identical. However, the proportions of each component were different. In the H30 fractions, the primary monosaccharide was xylose, followed by arabinose, glucose, uronic acid and galactose. However for the control sample, arabinose instead of xylose was the main monosaccharide and the galactose content was higher than that of uronic acid. By contrast, arabinose was the primary component of the H70 fractions, followed by uronic acid, xylose, glucose and galactose, except for the control sample where galactose content was higher than xylose. Smaller amounts of mannose, rhamnose and fucose were also detected.

The most significant changes in the monosaccharide composition of the coffee leaves following salt stress were observed for the H30 hemicellulosic fraction. Among the neutral monosaccharides, the content of xylose increased with salt stress, whereas those of arabinose, galactose, fucose and glucose were decreased after 25 days of salt stress. The concentrations of rhamnose and mannose were not different after 25 days of salt stress. However, salt stress increased the uronic acid concentration.

The HPSEC elution profiles of the H30 fractions are shown in Fig. 2C. A primary peak eluting at approximately 55 min and two other, less intense peaks, with elution times of approximately 47 and 61 min were detected in the control fraction. For the fraction extracted from plants subjected to salt stress, the peak eluting at the shorter retention time was shifted to higher molar mass values, and the intensity of the peaks eluting at approximately 55 and 61 min was decreased compared to the control.

The FTIR spectra of the H30 fractions (Fig. 3) showed glucuronoxylan bands at 1162 and 1043 cm^{-1} (Kačuráková et al., 2000), and the sizes of these bands were increased in the hemicellulosic fraction extracted from plants subjected to heat stress. These results are consistent with the increased levels of xylose and uronic acid observed for this fraction. In addition, the increase in xylose following salt stress was confirmed based on the increased size of the band at 1420 cm^{-1}, which is also related to the presence of xylans (Marga, Gallo, & Hasenstein, 2003).

As observed for the H30 fraction, the H70 fraction showed increased xylose and decreased arabinose contents in the plants subjected to salt stress compared to the control plants (Fig. 2B). For H30 fraction the increase of xylose and decrease of arabinose were observed in all sampling times (3–25 days). However, differently from H30 fraction, for the H70 fraction, after 25 days the arabinose content increased again to the values of the control plants.

Increases in the concentrations of rhamnose, mannose and uronic acid were observed following salt stress. However, after 25 days of salt stress, no differences were detected in the levels of these monosaccharides between the salt-stressed and control plants. By

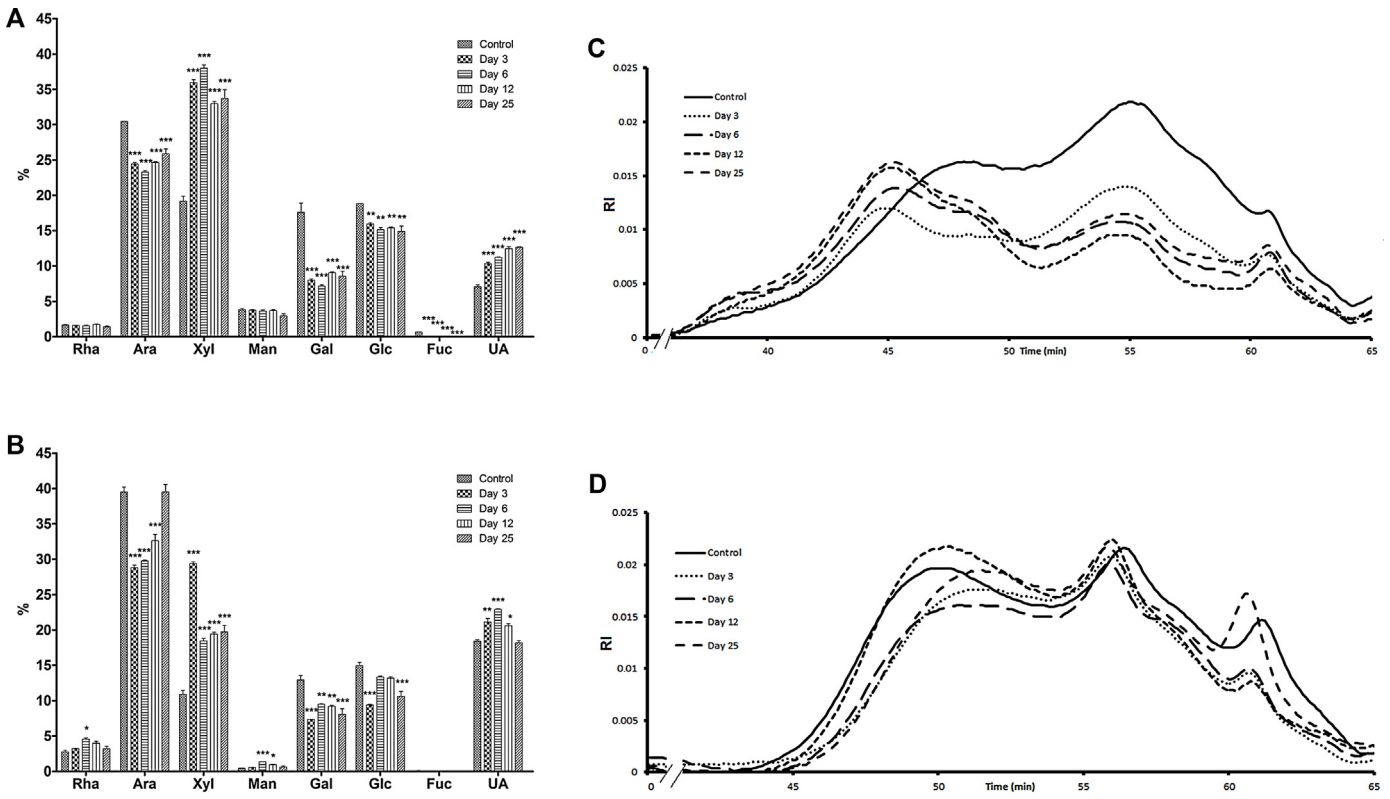

Fig. 2. Monosaccharide composition of the hemicellulosic fractions H30 (A) and H70 (B) from *Coffea arabica* control leaves and leaves subjected to salt stress; HPSEC analysis with RI detection for hemicellulosic fractions H30 (C) and H70 (D) from *Coffea arabica* control leaves and leaves subjected to salt stress. Rha, rhamnose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; Fuc, fucose, UA, uronic acid. Each value represents the mean of three determinations. The values are the mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

contrast, the levels of galactose and glucose decreased with salt stress.

The HPSEC elution profiles for the H70 fraction are shown in Fig. 2D. Similar to the H30 fraction, three primary peaks were observed. Peaks with elution times of approximately 49, 57, and 62 min were detected via RI for the control fraction. Following salt stress, changes in both the elution time and the proportion of peaks were observed. In the H70 fraction following salt stress, the peak with the shortest elution time was shifted toward lower molar mass values, which was different than what was observed in fraction H30. In addition, the peak with the longest elution time was shifted toward higher molar mass values when compared to the control. This result suggests that salt stress also caused changes in the hemicellulose contents of the cell walls of the coffee leaves.

3.4. Analysis of the final insoluble residue from cell walls of coffee leaves following saline stress

Glucose was the primary component of the final insoluble residues (Fig. 4A), indicating the presence of cellulose, as expected. Minor amounts of other monosaccharides from strong cross-linked non-cellulosic polysaccharides were also detected. In general, salt stress did not significantly alter the amount of monosaccharides observed.

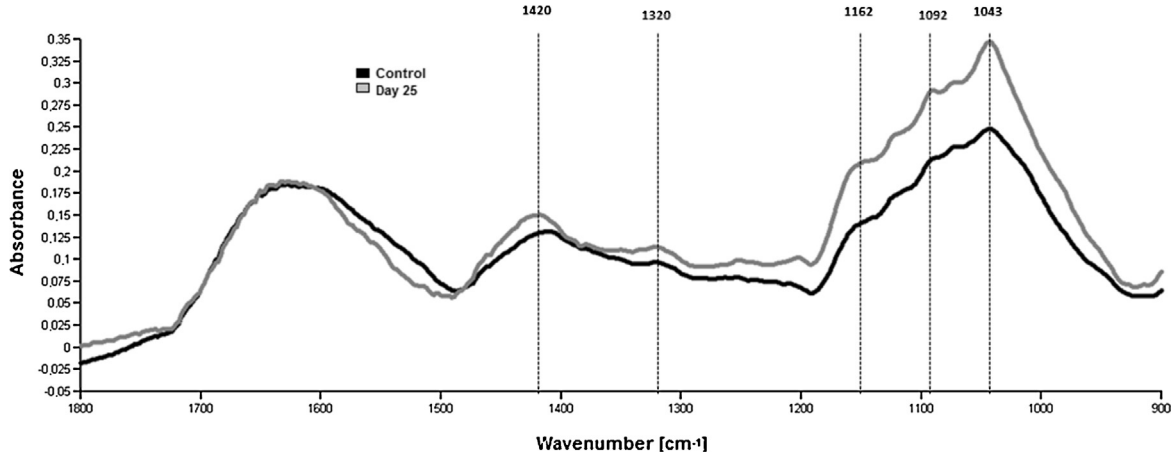

Fig. 3. FTIR spectra (control and after 25 days of salt stress) of the H30 fractions from *Coffea arabica* control leaves and leaves subjected to salt stress.

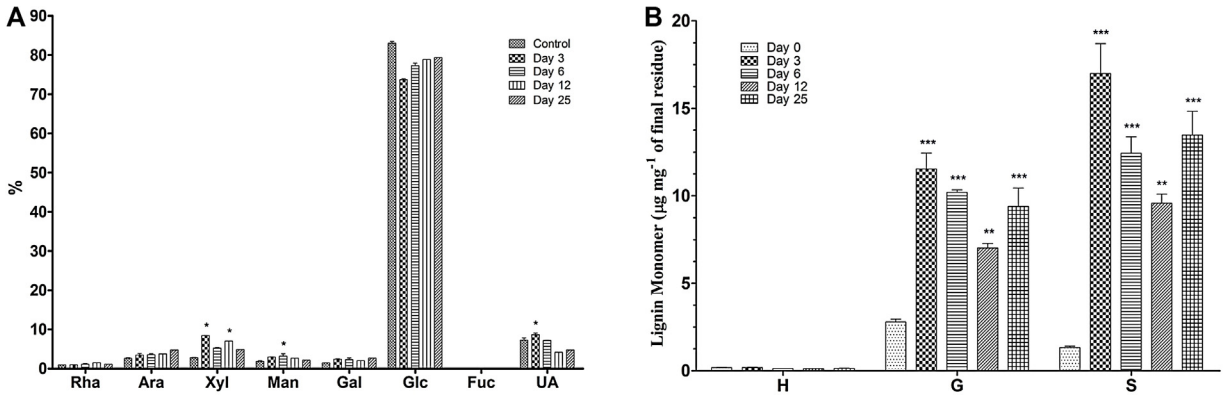

Fig. 4. Monosaccharide composition (A) and lignin monomer composition (B) of the final insoluble residues from *Coffea arabica* control leaves and leaves subjected to salt stress. Rha, rhamnose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; Fuc, fucose, UA, uronic acid; H, *p*-hydroxyphenyl; G, guaiacyl; S, Syringyl. Each value represents the mean of three determinations. The values are the mean ± S.E. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

In addition to cellulose, the insoluble cell wall material usually contains lignin (Li et al., 2010). As shown in Fig. 4B, analysis of the monolignol content in the final insoluble residue showed that the primary monolignols present were guaiacyl (G) and syringyl (S), with low amounts of *p*-hydroxyphenyl (H) being detected. Salt stress significantly increased the lignin monomer content of G and S compared to the control. This result suggests that salt stress induced an increase in the lignin levels in the cell walls of coffee leaves.

3.5. Analysis of the structure of coffee leaf cells following saline stress

The mesophyll of *C. arabica* is organized as a single layer consisting primarily of elongated columnar palisade parenchyma and a smaller proportion of spongy parenchyma cells that are irregularly shaped, thereby allowing gases to circulate through abundant air spaces (Fig. S1A and C). After 25 days of salt stress, the palisade parenchyma cells were more separated and thinner relative to the control (Fig. S1B and D). Consequently, the total thickness of the leaves was also thinner.

Clear ultrastructural differences in the cytoplasm and chloroplasts were observed between the control and 25 days of salt stress treatment (Fig. S2). The chloroplasts of the mesophyll cells in the control group displayed ellipsoidal shapes with well-developed membrane systems composed of grana and stroma and containing starch, and the chloroplast membrane was intact and not damaged (Fig. S2A and C). The granum-thylakoids and stroma-thylakoids exhibited an orderly arrangement, and there were dense stacks of grana, intergranal lamellae and a few plastoglobuli observed in the control plants. By contrast, after 25 days of NaCl treatment, the thylakoids in the self-grafted plants appeared to have swollen slightly, and the arrangement of the granum-thylakoids and stroma-thylakoids was disordered. Moreover, there was a decreased content of starch grains (Fig. S2B and D).

4. Discussion

The pectin and hemicellulose yields obtained through the sequential extraction of cell walls following abiotic stress have been evaluated to obtain information regarding the changes in the cell wall composition (Konno, Yamasaki, Sugimoto, & Takeda, 2008; Leucci, Lenucci, Piro, & Dalessandro, 2008; Wu & Cosgrove, 2000). The extractability of fractions W and H30 was decreased by salt stress (Table 1). These results may indicate changes in the organization of pectins and hemicelluloses in the cell wall. Similar results were obtained by Iraki, Bressan, Hasegawa, and Carpita (1989b) in tobacco cells subjected to salt and osmotic stresses. It was recently reported that the extractability of pectins and hemicellulose from coffee leaves is also decreased following heat stress treatment (Lima et al., 2013). The decreased yields of the W and H30 fractions observed after salt (Table 1) and heat stress (Lima et al., 2013) may be related to the establishment of stronger cross-linking between polysaccharides and cell wall stiffening. Thus making the extraction more difficult and consequently decreasing the yield of the polymers.

The high arabinose, galactose and uronic acid contents detected in the W fraction (Fig. 1A) from the leaves of control group plants were most likely released from the pectic polysaccharides of the primary cell wall, and the FTIR spectrum confirmed the presence of these components (Fig. 1C). Under salt stress, the uronic acid content increased, without alteration of the typical neutral pectic monosaccharides arabinose, galactose and rhamnose (Fig. 1A). Aquino, Grativol, and Mourão (2011) cultivated *Oryza sativa* plants in the presence or absence of 200 mM NaCl. The concentration of carboxylated polysaccharides was increased more than three-fold in the plants cultivated in the presence of salt. These authors proposed that negatively charged cell wall polysaccharides, such as pectins, may play a role in coping with salt stress.

The importance of the negatively charged cell wall polysaccharides in the salt stress was also observed for sulfated polysaccharides from halophytic species. It has been demonstrated that the sulfated polysaccharide concentration and the degree of sulfation in halophytic species are positively correlated with salinity (Aquino et al., 2011). The sulfated polysaccharides produced by *Ruppia maritima* Loisel have been observed to disappear when plants are cultivated in the absence of salt (Aquino et al., 2011). Although the function of sulfated polysaccharides in the plant cell wall remains undetermined, these authors proposed that the presence of sulfated polysaccharides might increase the Donnan potential because of the large negative charge associated with these polymers. This would increase the ion density in the vicinity of the plant cell wall, thereby facilitating ion transport at high salt concentrations. According to these authors, a similar mechanism may explain the function of pectin in the cell walls of plants exposed to high salinity conditions. On the other hand, it is also possible that the increase in negatively charged polysaccharides could contribute to slow the movement of Na^+ toward the cells. Na^+ is described to reach a toxic concentration before Cl^- (Carillo, Annunziata, Pontecorvo, Fuggi, & Woodrow, 2011).

Pectic side chains, such as arabinans, galactans and highly branched arabinogalactans of various configurations and sizes, are involved in determining the hydration status of the cell wall matrix due to their high water binding capability and ability to form gels

and establish the "pore size" of the pectin matrix as well as the diffusion of molecules through the wall (Willats, McCartney, Mackie, & Knox, 2001). In the present work, increased levels of uronic acid were observed in the pectic fractions after salt stress. However, no changes were detected in the arabinose and galactose contents of fractions isolated from plants submitted to salt stress. The increase in the uronic acid contents after the salt stress without changes in the arabinose and galactose contents could be due to hydrolysis of pectic polymers releasing short segments of homogalacturonans. This hypothesis is in agreement with the emergence of a peak eluting after 60 min in the HPSEC analysis of fractions obtained after the salt stress. In salt-treated roots, proteomic studies have identified different expression patterns of some glycosyl hydrolases which can be involved in cell remodeling (Zhao, Zhang, Wang, Chen, & Dai, 2013).

On the other side, the high levels of NaCl induce inhibition of the uptake of Ca^{2+} (Carillo et al., 2011) and Na^{+} could replace Ca^{2+} in the calcium–pectate interactions, thus losing the "egg-box" junctions and resulting in increased extractability of the homogalacturonans segments.

The hemicellulosic fractions H30 and H70 displayed high xylose contents. Cecy and Corrêa (1984) reported the presence of arabinoxylans, and Wenzel and Corrêa (1977) identified a 4-*O*-methyl-glucuronoxylan in the hemicelluloses of the leaves of *C. arabica* var Mundo Novo. The presence of xylose, arabinose and uronic acid as the main components of fractions H30 and H70, suggest that arabinoxylans and glucuronoxylans are present in the hemicellulosic fractions. The FTIR analysis also suggests the presence of arabinoxylans (Fig. 3). Fractions H30 and H70 also contain minor amounts of rhamnose probably due the presence of a fraction of rhamnogalacturonans which was tightly bonded to cell wall and was only extracted under more drastic extraction conditions. The H30 fractions also showed high contents of glucose and galactose, besides lower contents of fucose. The results suggest the presence of fucosylated xyloglucans which are typically found in primary cell walls of dicotyledonous species. After the stress, the levels of glucose and fucose were decreased, suggesting stronger cross-linking with cellulose in the cell wall, thus decreasing their extractability. The xyloglucan endotransglycosylase/hydrolase (XTHs) can hydrolyze and reform the bonds between xyloglucan chains to regulate cell wall rigidity. It has been shown that the overexpression of XTH3 gene in *Arabidopsis* and tomato enhanced tolerance to salt stress (Zhao et al., 2013).

The decrease in the arabinose content and the increase in xylose observed in the H30 and H70 fractions (Fig. 2A and B) following salt stress may be due to the loss of side chains from the arabinoxylans. Although, the arabinose content for the H70 fraction isolated after 25 days of salt stress was the same of the control.

A change in the degree of substitution of polysaccharides influences the cross-linking between celluloses and hemicelluloses. It is generally accepted that wall extensibility is limited by covalent bonds within the matrix polymers, which require enzymatic cleavage for wall loosening to occur (Burton, Gidley, & Fincher, 2010). Hemicellulosic polysaccharides with low degrees of substitution exhibit stronger cross-linking with cellulose microfibrils compared to those with high degrees of substitution (Carpita, 1996). Therefore, the low degree of arabinose substitution in the xylose backbone of arabinoxylans from H30 and H70 suggests stronger cross-linking between hemicellulose and cellulose in the cell walls, contributing to the stiffening of the cell wall which could enable plants to withstand high salinity stress. The lower degree of substitution of the arabinoxylans from H30 after the salt stress also decreases the solubility of this polymers. This is in agreement with the decrease in the yields of H30 fractions after the salt stress.

On the other side, after the salt stress the uronic acid contents were higher for both hemicellulosic fractions, H30 and H70 fractions, corroborating with the hypothesis that pointed to the importance of negatively charged cell wall polysaccharides in coping with salt stress. However, the uronic acid content of the H70 fraction isolated after 25 days was the same of the control. A reversion of the trend to values closer to the control was also observed for the arabinose content of the H70 fraction after 25 days of salt stress, which could be related with an adaptation to the stress conditions.

In addition to the decrease in arabinose substitution, the hemicellulosic fractions showed increased uronic acid contents following salt stress (Figs. 2A and 3A). This finding is consistent with the role of the negatively charged cell wall polysaccharides in coping with salt stress, as proposed by Aquino et al. (2011).

Beyond the observed changes in composition, the size of the hemicellulose fractions from coffee leaves increased under salt stress (Fig. 2C), also suggesting increased cross-linking between polysaccharides. It has been reported that tobacco cell cultures adapted to salt display an increased hemicellulose size (Iraki, Bressan, & Carpita, 1989a; Iraki, Singh, Bressan, & Carpita, 1989c) and thicker cell walls compared to unadapted cells (McCann, Shi, Roberts, & Carpita, 1994). Similarly, in the primary roots of cotton, salt stress causes an increase in the molecular size of the hemicellulosic fraction and the uronic acid content (Zhong & Läuchli, 1993).

The monomeric composition of lignin in the final insoluble residues showed high contents of G and S units, and these results correspond to those observed in other angiosperms (Boerjan, Ralph, & Baucher, 2003). Under salt stress, the monomer content was observed to increase in the present study (Fig. 4B). Plant cell walls are known to become lignified when the cell is under stress and when cells differentiate into particular specialized tissues, notably the xylem (Christensen, Bauw, Welinder, Moutagu, & Boerjan, 1998). Salinity stress has been associated with increased deposition of lignin in vascular tissues and/or xylem development. In bean-root vascular tissue, salt stress causes earlier and stronger lignification, which has been suggested to be a factor that inhibits growth and, consequently, represents an adaptation mechanism for resisting salinity-imposed stress (Cachorro, Ortiz, Barceló, & Cerdá, 1993). Heat stress has also been reported to increase the lignin content of coffee leaves (Lima et al., 2013). Moreover, changes in lignin contents following salt stress have been reported for soybean roots (*Glycine max*) (Neves, Marchiosi, Ferrarese, Siqueira-Soares, & Ferrarese-Filho, 2010) and beans (*Phaseolus vulgaris*) (Cachorro et al., 1993). Consistent with this hypothesis, two genes involved in lignin synthesis, S-adenosyl-L-methionine synthase (SAMS; EG967971) and caffeic acid 3-*O*-methyl-transferase (COMT; EG967224), have been found to be highly responsive to salt stress (Li et al., 2009). Different proteomic studies have indicated that cell wall lignification is important for plant salt tolerance (Zhao et al., 2013). It has been shown that lignification can also occur in the primary walls resulting in dramatic changes in the chemical and physical properties of plant cell walls (Schopfer, Lapierre, & Nolte, 2001). In the present work, the lignification of cell wall could also contribute to the stiffening of the cell wall as observed for the changes in the hemicellulose fractions, thus acting as a barrier for salt entrance.

Structural changes were observed in the mesophyll of the coffee leaves examined in the present work. It was observed that the palisade and spongy parenchyma exhibited thinner cells compared to the control plants (Fig. S1). These results may be related to the difficulty of the assimilation of water by the roots of the plants, which may be caused by osmotic stress due to the high amount of salt in the soil, thereby decreasing the water content in leaf cells.

The chloroplast performs photosynthesis and the anabolism of chlorophyll. Damage to the thylakoid membranes may inevitably lead to a significant decrease in photosynthesis (Zhang, Liu, Chang, & Anyia, 2010). In this study, the chloroplast structure was

visibly damaged by salt treatment in comparison to the control (Fig. S2). The chloroplast envelope was damaged, and the thylakoid was swollen; the granum-thylakoid and stroma-thylakoid were disordered; the granum and stroma lamellae were thin and obscure; and starch grains and plastoglobuli were detected (Fig. S2). This general damage is consistent with that described for the chloroplasts of cucumber seedlings under salt stress (Zhen, Bie, Huang, Liu, & Lei, 2011). The significant structural changes in the chloroplasts of the plant leaves are also likely caused by H_2O_2 oxidative damage because H_2O_2, a more stable ROS, can diffuse across biological membranes and cause oxidative protein modifications in areas distant from its production (Asada, 2006).

5. Conclusions

Structural damage to the cells of the mesophyll from leaves of *C. arabica* under salt stress was observed by electron and light microscopy. Changes in the extractability and size of polysaccharides isolated from cell walls of coffee leaves after salt stress suggest changes in the organization of pectins and hemicelluloses in the cell wall. According to the results, the cell walls of coffee leaves subjected to salt stress have undergone changes in the polysaccharide and lignin composition. These changes mainly result in stronger cross-linking between the cell wall polymers and the increase in negatively charged cell wall polysaccharides. The establishment of stronger cross-linking between polysaccharides and lignification of cell wall could contribute to the stiffening of the cell resulting in restriction of diffusion, thus acting as a barrier for salt entrance. The increase in the negatively charged cell wall polysaccharides has been pointed to be a role in coping with salt by facilitating ion transport at high salt concentrations. However, it is also possible that the negatively charged polysaccharides act delaying the entrance of Na^+.

Acknowledgements

The authors thank the Brazilian agencies CNPq, CAPES and Fundação Araucária-PRONEX, for their financial support and the Laboratório Central de Microscopia Eletrônica -UFSC (LCME-UFSC) for the facilities and technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.06.042.

References

Ahmed, A. E. R., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry*, *1*, 361–365.
Albersheim, P., An, J., Freshour, G., Fuller, M. S., Guillen, R., Ham, K.-S., et al. (1994). Structure and functions studies of plant cell wall polysaccharides. *Biochemical Society Transactions*, *22*, 374–378.
Albini, F. M., Murelli, C., Patriti, G., Rovati, M., Zienna, P., & Finzi, P. V. (1994). Low-molecular weight substances from the resurrection plant *Sporobolus stapfianus*. *Phytochemistry*, *37*, 137–142.
Aquino, R. S., Grativol, C., & Mourão, P. A. S. (2011). Rising from the sea: Correlations between sulfated polysaccharides and salinity in plants. *PLoS ONE*, *6*(4), 1–7.
Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, *141*(2), 391–396.
Bacic, A. (2006). Breaking an impasse in pectin biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(15), 5639–5640.
Bennici, A., & Tani, C. (2009). Ultrastructural effects of salinity in *Nicotiana bigelovii* var. bifelovii callus cells and *Allium cepa* roots. *Caryologia*, *62*(2), 124–133.
Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.
Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. *Annual Review of Plant Biology*, *54*(1), 519–546.
Brett, C., & Waldron, K. (1990). *Physiology and biochemistry of plant cell walls*. London: Springer.
Budzinski, I. G. F., Santos, T. B., Sera, T., Pot, D., Vieira, L. G. E., & Pereira, L. F. P. (2010). Expression patterns of three α-expansin isoforms in *Coffea arabica* during fruit development. *Plant Biology*, *13*(3), 462–471.
Burton, R. A., Gidley, M. J., & Fincher, G. B. (2010). Heterogeneity in the chemistry, structure and function of plant cell walls. *Nature Chemical Biology*, *5*, 724–732.
Cachorro, P., Ortiz, A., Barceló, A. R., & Cerdá, A. (1993). Lignin deposition in vascular tissues of *Phaseolus vulgaris* tissues of *Phaseolus vulgaris* roots in response to salt stress and Ca^{2+} ions. *Phyton (Horn, Austria)*, *33*(1), 33–40.
Cardemil, L., & Riquelme, A. (1991). Expression of cell wall protein in seeds and during early seedling growth of *Araucaria araucana* is a response to wound stress and is developmentally regulated. *Journal of Experimental Botany*, *42*, 415–421.
Carillo, P., Annunziata, M. G., Pontecorvo, G., Fuggi, A., & Woodrow, P. (2011). Salinity stress and salt tolerance. In A. Shanker (Ed.), *Abiotic stress in plants – Mechanisms and adaptations*. In Tech. http://dx.doi.org/10.5772/22331. ISBN: 978-953-307-394-1, Available from: http://www.intechopen.com/books/abiotic-stress-in-plants-mechanisms-and-adaptations/salinity-stress-and-salt-tolerance
Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 445–476.
Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, *3*, 1–30.
Cecy, I. I. T., & Corrêa, J. B. C. (1984). Hemicellulosic polymers from the leaves of *Coffea arabica*. *Phytochemistry*, *23*(6), 1271–1276.
Christensen, J. H., Bauw, G., Welinder, K. G., Moutagu, M. V., & Boerjan, W. (1998). Purification and characterization of peroxidases correlated with lignification in poplar xylem. *Plant Physiology*, *118*, 125–135.
Doblin, M. S., Pettolino, F., & Bacic, A. (2010). Plant cell walls: The skeleton of the plant world. *Functional Plant Biology*, *37*, 357–381.
Domon, J.-M., Baldwin, L., Acket, S., Caudeville, E., Arnoult, S., Zub, H. M., et al. (2013). Cell wall compositional modifications of *Miscanthus* ecotypes in response to cold acclimation. *Phytochemistry*, *85*, 51–61.
Dos Santos, T. B., Budzinski, I. G. F., Marur, C. J., Petkowicz, C. L. O., Pereira, L. F. P., & Vieira, L. G. E. (2011). Expression of three galactinol synthase isoforms in *Coffea arabica* L. and accumulation of raffinose and stachyose in response to abiotic stresses. *Plant Physiology and Biochemistry*, *49*, 441–448.
Feder, N., & O'Brien, T. P. (1968). Plant microtechnique: Some principles and new methods. *American Journal of Botany*, *55*(1), 123–142.
Fernandes, J. C., Garcia-Angulo, P., Goulao, L. F., Acebes, J. L., & Amancio, S. (2013). Mineral stress affects the cell wall composition of grapevine (*Vitis vinifera* L.) callus. *Plant Science*, *205-206*, 111–120.
Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*, *55*, 307–319.
ICO – International Coffee Organization (2014). Available on http://www.ico.org/prices/m1.htm
Iraki, N. M., Bressan, R. A., & Carpita, N. C. (1989). Extracellular polysacharides and proteins of tobacco cell cultures and changes in composition associated with growth-limiting adaptation to water and saline stress. *Plant Physiology*, *91*, 54–61.
Iraki, N. M., Bressan, R. A., Hasegawa, P. M., & Carpita, N. C. (1989). Alteration of the physical and chemical structure of primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiology*, *91*, 39–47.
Iraki, N. M., Singh, N., Bressan, R. A., & Carpita, N. C. (1989). Cell walls of tobacco cells and changes in composition associated with reduced growth upon adaptation to water and saline stress. *Plant Physiology*, *91*, 48–53.
Kačuráková, M., Capek, P., Sasinková, V., Wellner, N., & Ebringerová, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, *43*, 195–203.
Karnovsky, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, *27*, 137–138.
Kerepesi, I., & Galiba, G. (2000). Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. *Crop Science*, *40* (482-487)
Konno, H., Yamasaki, Y., Sugimoto, M., & Takeda, K. (2008). Differential changes in cell wall matrix polysaccharides and glycoside-hydrolyzing enzymes in developing wheat seedlings differing in drought tolerance. *Journal of Plant Physiology*, *165*(7), 745–754.
Leucci, M., Lenucci, M., Piro, G., & Dalessandro, G. (2008). Water stress and cell wall polysaccharides in the apical root zone of wheat cultivars varying in drought tolerance. *Journal of Plant Physiology*, *165*(11), 1168–1180.
Li, H., Wang, Y., Jiang, J., Liu, G., Gao, C., & Yang, C. (2009). Identification of genes responsive to salt stress on *Tamarix hispida* roots. *Gene*, *433*(1–2), 65–71.
Li, X., Ximenes, E., Kim, Y., Slininger, M., Meilan, R., Ladisch, M., et al. (2010). Lignin monomer composition affects *Arabidopsis* cell-wall degradability after liquid hot water pretreatment. *Biotechnology for Biofuels*, *3*(1), 27.
Lima, R. B., dos Santos, T. B., Vieira, L. G. E., Ferrarese, M. d. L. L., Ferrarese-Filho, O., Donatti, L., et al. (2013). Heat stress causes alterations in the cell-wall polymers and anatomy of coffee leaves (*Coffea arabica* L.). *Carbohydrate Polymers*, *93*(1), 135–143.
Luft, J. H. (1961). Improvements in epoxy resin embedding methods. *Journal of Biophysical and Biochemical Cytology*, *9*, 409–414.
Marga, F., Gallo, A., & Hasenstein, K. H. (2003). Cell wall components affect mechanical properties: Studies with thistle flowers. *Plant Physiology and Biochemistry*, *41*(9), 792–797.
McCann, M. C., Shi, J., Roberts, K., & Carpita, N. C. (1994). Changes in pectin structure and localization during the growth of unadaped and NaCl-adapted tobacco cells. *Plant Journal*, *5*, 773–785.

Miyake, H., Mitsuya, S., & Rahman, M. S. (2006). Ultrastructural effects of salinity stress in higher plants. In A. Rai, & T. Takabe (Eds.), *Abiotic stress tolerance in plants* (pp. 215–226). Netherlands: Springer.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*(1), 651–681.

Nazário, A. A., Garcia, G. D. O., Gonçalves, I. Z., Madalão, J. C., & Araujo, G. L. (2010). Growth conilon coffee irrigated with saline water. *Engenharia Ambiental – Espírito Santo do Pinhal*, *7*(3), 178–195.

N'Diaye, S., & Rigal, L. (2000). Factors influencing the alkaline extraction of poplar hemicelluloses in a twin-screw reactor: Correlation with specific mechanical energy and residence time distribution of the liquid phase. *Bioresource Technology*, *75*, 13–18.

Neves, G. Y. S., Marchiosi, R., Ferrarese, M. L. L., Siqueira-Soares, R. C., & Ferrarese-Filho, O. (2010). Root growth inhibition and lignification induced by salt stress in soybean. *Journal of Agronomy and Crop Science*, *196*, 467–473.

Nishizawa, A., Yabuta, Y., & Shigeoka, S. (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology*, *147*(3), 1251–1263.

Pennell, R. (1998). Cell walls: Structures and signals. *Current Opinion in Plant Biology*, *1*, 504–510.

Recio, E. (2003). Autolysis-like release of homogalacturonan from bean (*Phaseolus vulgaris* L.) callus cell walls. *Plant Science*, *164*(4), 579–588.

Schopfer, P., Lapierre, C., & Nolte, T. (2001). Light-controlled growth of the maize seedling mesocotyl: Mechanical cell-wall changes in the elongation zone and related changes in lignification. *Physiologia Plantarum*, *111*, 83–92.

Taiz, L., & Zeiger, E. (2006). *Plant Physiology*. Sunderland, MA: Sinauer Associates, Inc., Publishers.

Vinogradov, E., & Wasser, S. (2005). The structure of a polysaccharide isolated from *Inonotus levis* P. Karst. mushroom (Heterobasidiomycetes). *Carbohydrate Research*, *340*(18), 2821–2825.

Wakabayashi, K., Hoson, T., & Kamisaka, S. (1997). Osmotic stress suppresses cell wall stiffening and the increase in cell wall-bound ferulic and diferulic acids in wheat coleoptiles. *Plant Physiology*, *113*, 967–973.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, *218*(1), 1–14.

Weiser, R. L., Wallner, S. J., & Waddell, J. W. (1990). Cell wall and extensin mRNA changes during cold acclimation of pea seedlings. *Plant Physiology*, *93*, 1021–2026.

Wenzel, G. E., & Corrêa, J. B. C. (1977). Fractionation of the hemicellulose and structural analysis of 4-O-methylglucuronoxylan. *Anais da Academia Brasileira de Ciências*, *49*, 605–613.

Willats, W. G. T., McCartney, L., Mackie, W., & Knox, J. P. (2001). Pectin: Cell biology and prospects for functional analysis. *Plant Molecular Biology*, *47*, 9–27.

Wilson, R. H., Smith, A. C., Kacuráková, M., Saunders, P. K., Wellner, N., & Waldron, K. W. (2000). The mechanical properties and molecular dynamics of plant cell wall polysaccharides studied by Fourier-transform infrared spectroscopy. *Plant Physiology*, *124*, 397–405.

Wu, Y., & Cosgrove, D. J. (2000). Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *Journal of Experimental Botany*, *51*, 1543–1553.

Zanardo, D. I. L., Lima, R. B., Ferrarese, M. d. L. L., Bubna, G. A., & Ferrarese-Filho, O. (2009). Soybean root growth inhibition and lignification induced by *p*-coumaric acid. *Environmental and Experimental Botany*, *66*(1), 25–30.

Zhang, B., Liu, W., Chang, S. X., & Anyia, A. O. (2010). Water-deficit and high temperature affected water use efficiency and arabinoxylan concentration in spring wheat. *Journal of Cereal Science*, *52*(2), 263–269.

Zhao, Q., Zhang, H., Wang, T., Chen, S., & Dai, S. (2013). Proteomics-based investigation of salt-responsive mechanisms in plant roots. *Journal of Proteomics*, *82*, 230–253.

Zhen, A., Bie, Z., Huang, Y., Liu, Z., & Lei, B. (2011). Effects of salt-tolerant rootstock grafting on ultrastructure, photosynthetic capacity, and H_2O_2-scavenging system in chloroplasts of cucumber seedlings under NaCl stress. *Acta Physiologiae Plantarum*, *33*, 2311–2319.

Zhong, H., & Läuchli, A. (1993). Changes of cell wall composition and polymer size in primary roots of cotton seedlings under high salinity. *Journal of Experimental Botany*, *44*, 773–778.

Zwiazek, J. J. (1991). Cell changes in white spruce (*Picea glauca*) needles subjected to repeated drought stress. *Physiologia Plantarum*, *82*, 513–518.